The Use of 6-Benzylaminopurine for Rapid Multiplication of Tillandsias

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Abstract. Some species of the genus *Tillandsia* (Bromeliaceae) are highly endangered in their natural habitats. This report introduces a method for induced lateral shoot development in atmospheric Tillandsias using the cytokinin BAP. Spraying or dipping the plants with BAP-solutions induced many lateral shoots. Even difficult-to-propagate species like *T. cacticola* L.B.Sm developed up to 30 times more usable shoots than the control. Applying low concentrations (1 and 5 mg·L⁻¹) over a longer period of time (10 weeks, 3 times a week) produced results similar to those using higher concentrations (50 mg·L⁻¹) for shorter periods (2 weeks, 3 times a week). Chemical name used: 6-benzylaminopurine (BAP).

The horticultural propagation of various species of the genus *Tillandsia* is slow. Under European conditions, it takes 4 to 5 years after sowing before the plants are big enough for sale or until flower induction is possible. Vegetative propagation via lateral shoot formation is possible and usually used. After an inflorescence develops from the apical region, some shoots start to emerge from lateral meristems, but the number of these shoots is often small, depending on the species and on the growing conditions. Sideshoot formation is affected by apical dominance. Decapitation enables the plant to develop shoots from lateral meristems, although few lateral shoots are produced.

Mass propagation in vitro is also possible (Labus-Schneider and Abel, 1991; Zimmer et al., 1993).

Early investigations with *T. aeranthos* (Loisel.) L. B. Sm. showed high potential for rapid multiplication of atmospheric Tillandsias (= epiphytic Tillandsias with the possibility of water and nutrition-uptake through the leaves) by external application of the cytokinin BAP (Bessler and Zimmer, 1993). The use of BAP for lateral shoot development is already described in some propagation-systems of ornamental plants (Carpenter and Beck, 1972; Carpenter and Carlson, 1972a, 1972b; Harkess and Lyons, 1994; Jeffcoat, 1977; Richards and Wilkinson, 1984; Sieminska-Michalek, 1989), but little is known about its use in Bromeliad propagation (Bessler and Zimmer, 1993; Billenstein and Ritter, 1983; Gardner, 1977; Pytlewski, 1989; Stoutemeyer, 1976).

Looking at the enormous number of atmospheric Tillandsias imported from South America to Europe and the United States raises the question of the origin of the imported

plants. Some of these species are highly endangered and trade is regulated by the Convention on International Trade in Endangered Species (CITES). Some nurseries for Tillandsia production exist, but sometimes, because of slow growth and low multiplication-rates, it is easier to collect native plants for greenhouse rearing prior to sale.

This report describes a simple procedure for rapid multiplication of atmospheric Tillandsias by hormonal induction of lateral-shoot formation.

Materials and Methods

Three species of *Tillandsia* having differing propagation characteristics [*T. butzii* Mez., many lateral shoots, easy to propagate; *T. cacticola*, only few shoots after flowering, slow growth and development, difficult to propagate; *T. aeranthos*, intermediate propagation possibilities (Bessler and Zimmer, 1993)] were used for the experiments. All plants were grown in a standard greenhouse (20 °C, 12 h additional light additional to natural daylight during the day, high-pressure sodium lamps, ≈45 μmol·m⁻²·s⁻¹) and were developed from in vitro culture (*T. butzii*, *T. cacticola*) or lateral-shoot formation in situ (*T. aeranthos*).

For standardization of the material I assumed that each leaf axil bears one lateral meristem and is, therefore, a potential site for lateral-shoot development and thus reflects the initial number of leaves (Table 1).

Concentration, mode of application, and frequency of application of BAP were variables. Sprayed solutions were applied with a hand sprayer until runoff. *Tillandsia butzii* were packed in perforated plastic bags before being dipped into the solutions. *Tillandsia aeranthos* was investigated to determine if the age of the meristem influenced multiplication. Plants (≈8 years old, 25 cm long, one single shoot) were cut into three similar sections, (apical, center, basal) each with a similar number of leaves (25–30) and treated as described in Table 1.

BAP (Sigma Chemical Co., Deisenhofen, Germany) was dissolved in a few drops of 1 N NaOH and mixed with water. The stock solution, neutralized with 1 N HCl and having a final ratio of 1:1 (1 mL = 1 mg) was diluted with water to the concentrations used in the experiments (spraying and dipping, Table 1). One drop of Tween 80 per liter was used as a surfactant. The number of newly developed lateral shoots was counted at various times after the last application of BAP (Table 1).

The significance of treatment effects was determined using analysis of variance. Variation between treatment means was analyzed using Tukey's procedure. The significance of the correlation was analyzed by calculating the correlation coefficient *r*.

Results and Discussion

Concentration and frequency of application of BAP. Generally, the application of BAP enhanced the development of lateral shoots. The highest mean number of lateral shoots for the difficult-to-propagate species *T. cacticola* was obtained by using BAP at 50 mg·L⁻¹ (Fig. 1), which was the highest concentration tested. Even using the lowest concentration (1 mg·L⁻¹) resulted in a higher yield of usable lateral shoots than the control. BAP at 50 mg·L⁻¹ applied for 8 or 10 weeks led to the formation of more than one shoot in some axils. Before these multiple shoots developed, some swelling of the lateral meristems was visible.

Results were similar for the easy-to-propagate species *T. butzii* (Table 2). The highest number of lateral shoots developed after spraying the plants for a few times with high concentrations or more often with low concentrations of BAP. Spraying the plants 6 times with BAP at 25 mg·L⁻¹ solution resulted in a harvest of 5.1 (±1.1) lateral shoots, compared to 2.8 (±1.0) for the control.

These results agree with Bessler and Zimmer's (1993) with *T. aeranthos*. This response appears to be a dose-dependent reaction with concentration and frequency of application playing an important role in this rapid multiplication system.

Since multiple sprays with high concentrations of BAP solutions is expensive, the second experiment investigated the possibility of dipping *T. butzii* plants into a single concentration of solution. All plants that were dipped for 24 h, whether 1, 2, or 3 times, died shortly after these treatments. All other treatments led to a significantly higher number of shoots than the control. Even putting the plants once for 1 h into the cytokinin solution resulted in more than double the number of usable shoots, compared with the control (Table 3). Considering that plants having seven leaves were selected for this experiment, the average number of shoots (up to 5.9) is very high.

Influence of the age (position) of the meristem. It is important to know if the potential for regeneration of lateral shoots changes as plants age. BAP was sprayed on lateral meristems of *T. aeranthos* from several positions, thus, presumably, on various-aged meristems (Table 4). Shoots were counted 50, 100, and

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Table 1. Details of the experiments on improving propagation of three *Tillandsia* species.

Details	Species			
	<i>T. butzii</i>	<i>T. butzii</i>	<i>T. aeranthos</i>	<i>T. cacticola</i>
BAP (mg·L ⁻¹)	0, 1, 5, 10, 25	10	0, 1, 5, 10, 25	0, 1, 5, 10, 25, 50
Application frequency	3 times a week	1, 2, 3 times	3 times a week	3 times a week
Duration	2, 4, 6, 8, 10 weeks	1, 8, 24 h	4, 8 weeks	2, 4, 6, 8, 10 weeks
Initial leaf count	7	7	25–30	24
Mode	Spray	Dipping	Spray	Spray
Interval (days) ^z	105	105	50, 100, 150	105
Plants (no.) ^y	15	26	10, 12	12
Results in	Table 2	Table 3	Table 4	Fig. 1

^zTime between last treatment and evaluation.^yPer treatment. Fig. 1. Effect of various BAP treatments on mean number of lateral shoots developed by *Tillandsia cacticola*. Each point represents a sample from 12 plants. Regression model: $y = 0.19x + 4.4$; $n = 26$; $r = 0.84$, $r^2 = 0.71$; calculated by using the shown mean.

150 days after the last BAP application. BAP at 5 and 10 mg·L⁻¹ must be applied for 8 weeks, 4 weeks of application resulted in fewer lateral shoots (Table 4). Even ≈8-year-old dormant lateral meristems were stimulated to develop lateral shoots after treatment with BAP; however, development was slow. Thus, results are shown only for day 150; further counts were not possible because some of the newly initiated lateral shoots began to flower.

All the results indicate the high potential of using BAP as a multiplication agent in atmospheric Tillandsias. Reports of use on other ornamental plants indicated problems in root formation of the induced lateral shoots in plant production via cuttings (Carpenter and Beck, 1972; Carpenter and Carlson, 1972b). Atmospheric Tillandsias do not need any roots when produced for horticultural uses. The roots in these species play an important role during germination and later in the natural habitat as these bromeliads are epiphytes and adhere to a host. All nutrition and uptake of water occurs through specialized cells in the leaves. This adaptation allows atmospheric Tillandsias to be propagated as described.

BAP is difficult to transport to the lateral meristems. Exogenous application of BAP does not ensure endogenous translocation (Moore, 1989), suggesting that meristem development into shoots requires direct contact

with the BAP solution. Other synthetic cytokinins, like 6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purin (PBA), are said to translocate better inside the plant (Wang, 1988) and might achieve better results than BAP. Early experiments using 2,3,5 triiodobenzoic acid (TIBA) as a substance that inhibits the transport of

auxins from apical to basal regions did not result in more lateral shoot in the tested Tillandsias compared with applying BAP alone or in combination with TIBA (T. Jonas, personal communication).

All results showed the potential of using BAP as an aid in the development of a rapid multiplication system for atmospheric Tillandsias. Using it in nurseries close to their natural habitats or in Europe and the United States might prevent the possible depredation of native Tillandsias.

Literature Cited

- Bessler, B. and K. Zimmer 1993. Vegetative Vermehrung von "atmosphärischen" Tillandsien. I. Einfluß von Benzylaminopurin auf die Seitentriebbildung von *T. aeranthos*. Gartenbauwissenschaft 58:117–120.
- Billenstein, H. and R. Ritter, 1983. On the vegetative propagation of orchids. South African Orchid J. 14:7.
- Carpenter, W.J. and G.R. Beck 1972. Growth regulator induced branching of poinsettia stock plants. HortScience 7:405–406.
- Carpenter, W.J. and W.H. Carlson 1972a. Improved *Geranium* branching with growth regulator sprays. HortScience 7:291–292.
- Carpenter, W.J. and W.H. Carlson 1972b. The effect of growth regulators on *Chrysanthemum*. J. Amer. Soc. Hort. Sci. 97:349–351.
- Gardner, S. 1977. Induction of lateral growth on *Vriesea* by cytokinin. J. Bromeliad Soc. 27:31–33.

Table 2. Effect of various BAP applications on the mean number of lateral shoots in *Tillandsia butzii*.

BAP (mg·L ⁻¹)	Lateral shoots (mean no.)				
	No. applications				
	6	12	18	24	30
0					2.8 k ^z , y
1	2.3 l	3.2 h–k	3.3 h–j	4.4 b–d	3.5 f–i
5	3.1 i–k	3.8 e–g	3.5 f–i	4.6 bc	3.4 g–i
10	3.9 ef	4.0 de	2.9 jk	3.6 e–h	3.4 g–i
25	5.1 a	4.8 ab	4.0 de	3.8 e–g	3.2 h–k

^zControl.^yMean separations within rows and columns by Tukey's test at $P \leq 0.05$.Table 3. Effect of BAP treatments (submergence in a solution, BAP at 10 mg·L⁻¹) on the mean number of lateral shoots in *Tillandsia butzii*.

Duration of submergence (h) ^z	Lateral shoots (mean no.)		
	No. treatments		
	1	2	3
0			2.2 a ^y , x
1	4.7 b	5.0 bc	5.4 c
8	5.3 c	5.9 d	5.2 c

^zAll plants died after submerging for 24 h.^yControl.^xMean separations within rows and columns by Tukey's test at $P \leq 0.05$.

Table 4. Effect of various BAP treatments and age (position) of the lateral meristems on the mean number of lateral shoots in *Tillandsia aeranthos*.

Part	Lateral shoots (mean no.)				
	BAP (mg·L ⁻¹)				
	0	1	5	10	25
<i>12 Applications</i>					
Apical	3.5	3.3	4.1	9.5	11.2
Central	2.3	2.9	2.6	4.8	6.8
Basal	2.4	2.7	1.8	2.4	3.7
<i>24 Applications</i>					
Apical	3.4	3.2	11.7	13.8	11.8
Central	2.8	3.0	4.3	6.5	8.8
Basal	2.3	2.3	4.4	4.0	2.6

Table 5. Table of significance for Table 4.

Variable	df	Mean square	Significance of F
Number of applications (N)	1	149.9	***
Concentration (C)	4	302.6	***
Part (P)	2	551.6	***
N × C	4	44.7	***
N × P	2	21.3	NS
C × P	8	75.7	***
N × C × P	8	13.6	NS
Error	295	10.6	

NS, ***Nonsignificant or significant at $P \leq 0.001$, respectively, by F test.

Harkess, R.L. and R.E. Lyons 1994. Gibberellin- and cytokinin-induced growth and flowering responses in *Rudbeckia hirta* L.. HortScience 29:141-142.

Jeffcoat, B. 1977. Influence of the cytokinin 6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purine on the growth and development of some ornamental crops. J. Hort. Sci. 52:143-153

Labus-Schneider, F.O. and W.O. Abel 1991. Regeneration of *Tillandsia* through immature embryo culture. Acta Hort. 289:121-122.

Moore, T.C. 1989. Biochemistry and physiology of plant hormones. 2nd ed. Springer, New York

Pytlewsky, C. 1989. The practical possibilities of the use of BA in vegetative propagation on the *Cryptanthus* genus. Acta Hort. 251:353-358.

Richards, D. and R.I. Wilkinson 1984. Effect of manual pinching, potting-on, and cytokinins on branching and flowering of *Camellia*, *Rhododendron* and *Rosa*. Scientia Hort. 23:75-83.

Sieminska-Michalek, E. 1989. Influence of growth regulators on branching and elongation of pot plants. Acta Hort. 251:341-343.

Stoutemeyer, V. 1976. Cytokinins and the propagation of bromeliads. J. Bromeliad Soc. 26:197

Wang, Y-T. 1988. Branching agents. Greenhouse Manager 7:136-141.

Zimmer, K., B. Bessler, and D. Vett 1993. Vegetative Vermehrung von "atmosphärischen Tillandsien" I. in-vitro-Vermehrung. Gartenbauwissenschaft 58:164-169.